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INFORMATION DISCLOSURE STATEMENT

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Washington, D.C. 20231

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By: Diane E. Ingolia
Diane E. Ingolia

Sir or Madam:

The citations listed below, copies attached, may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97. The Examiner is requested to make these citations of official record in this application.

The following patents are referred to in the body of the specification.

- U.S. Patent No. 4,683,195 issued to Mullis;
- U.S. Patent No. 4,683,202 issued to Mullis *et al.*;
- U.S. Patent No. 5,108,892 issued to Burke *et al.*;
- U.S. Patent No. 5,144,019 issued to Rossi *et al.*;
- U.S. Patent No. 4,511,502 issued to Builder & Ogez;
- U.S. Patent No. 4,518,526 issued to Olson;

- U.S. Patent No. 4,511,503 issued to Olson & Pai;
- U.S. Patent No. 4,512,922 issued to Jones *et al.*;
- PCT Application No. W0 90/01069 A1 to Segev;
- PCT Application No. WO 92/06200 to Gelfand *et al.*;
- PCT Application No. WO 91/09950 to Gelfand *et al.;*
- PCT Publication WO 90/15157 to Lane *et al.*.

The following printed publications are referred to in the body of the specification:

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- Widjojoatmondjo *et al.*, "Rapid Identification of Bacteria by PCR-Single-Strand Conformation Polymorphism," J. Clin. Microbiol. 32:3002 (1994);
- Maidak *et al.*, "The Ribosomal Database project," Nucleic Acids Res., 22:3485 (1994);
- McConlogue *et al.*, "Structure-independent DNA amplification by PCR using 7-deaza-2'-deoxyguanosine," Nucleic Acids Res. 16:20 (1988);

Applicants have become aware of the following printed publications which they believe may be material to the examination of the instant application:

- U.S. Patent No. 5,422,253 to Dahlberg *et al.* describes methods for the cleavage of a single-stranded target nucleic acid molecule by forming a cleavage structure comprising the target nucleic acid strand and a pilot nucleic acid (*i.e.*, an oligonucleotide). Cleavage of nucleic acids at regions of intramolecular annealing in the absence of pilot oligonucleotides is also described. In contrast to the claimed invention, this reference does not describe the cleavage of nucleic acid substrates derived from microorganisms to produce a characteristic set of cleavage products.
- U.S. Patent No. 5,541,311 to Dahlberg *et al.* describes the generation of DNA sequences encoding modified DNA polymerases that retain 5' nuclease activity but lack synthetic activity and the use of these modified DNA polymerases for the detection of specific nucleic acid sequences in an assay employing cleavage structures formed by annealing a target nucleic acid with one or more oligonucleotides. In contrast to the claimed invention, this reference does not describe the cleavage of nucleic acid substrates derived from microorganisms to produce a characteristic set of cleavage products;

- U.S. Patent No. 5,614,402 to Dahlberg *et al.* describes methods for the detection of specific nucleic acid sequences in an assay employing modified DNA polymerases lacking synthetic activity and cleavage structures formed by annealing a target nucleic acid with one or more oligonucleotides. In contrast to the claimed invention, this reference does not describe the cleavage of nucleic acid substrates derived from microorganisms to produce a characteristic set of cleavage products;
- D.S. Sigman *et al.*, "Chemical Nucleases," *Chemical Reviews* 93:2295-2316 (1993) describe the use of chemical (*i.e.*, non-enzymatic) nucleases for the cleavage of nucleic acids. In contrast, the claimed invention specifies that an enzymatic cleavage means is used to cleave a nucleic acid substrate containing cleavage structures (*i.e.*, single strands of nucleic acids containing a region(s) of secondary structure). Sigman *et al.* fail to teach the enzymatic cleavage of nucleic acid substrates containing cleavage structures;
- T.R. Cech *et al.*, "Secondary Structure of the *Tetrahymena* ribosomal RNA intervening sequence, Structural homology with fungal mitochondrial intervening sequences," *Proc. Natl. Acad. Sci.* USA 80:3903 (1983) report the cleavage of a single wild-type RNA substrate (the *Tetrahymena* IVS RNA) using nucleases specific for the cleavage of RNA substrates (*i.e.*, RNases). Cech *et al.* do not teach the cleavage of a nucleic acid substrate followed by the comparison of the resulting cleavage products with a reference control nor the formation of cleavage structures by heating and cooling of a nucleic acid substrate nor the extraction of nucleic acids from a sample followed by cleavage of cleavage structures. Further, Cech *et al.* do not teach the use of nucleases in a solution containing manganese;

- C.R. Woese *et al.*, "Detailed Analysis of the Higher Order Structure of 16S Like Ribosomal Ribonucleic Acids," *Microbilogy Reviews* 47:621 (1983) provides a review summarizing evidence for supporting proposed secondary structures for 16S-like ribosomal RNAs. Woese *et al.* teach that the primary sequences of corresponding rRNAs are compared in order to confirm the predicted secondary structures of those rRNAs. In contrast to the claimed invention, Woese *et al.* do not teach the cleavage of a nucleic acid substrate containing cleavage structures to generate a characteristic set of cleavage products. Woese *et al.* further do not teach the cleavage of cleavage structures using 5' nucleases derived from DNA polymerases nor the formation of cleavage structures by heating and cooling of a nucleic acid substrate;
- Hoheisel *et al.*, "On The Activities of *Escherichia coli* Exonuclease III," *Anal. Biochem.* 209:238-246 (1993). Hoheisel *et al.* disclose the results of a study of the exonuclease activity of the *E. coli* exonuclease III enzyme. Double stranded nucleic acid sequences which contain an overhang at the 3'-end of up to 4 nt long are cleaved by the enzyme. The disclosure by Hoheisel *et al.* is distinguished from the claimed invention in that Hoheisel *et al.* does not disclose treating the nucleic acid substrate under conditions such that single stranded nucleic acid sequences form a secondary structure(s). Rather, the single stranded overhangs disclosed by Hoheisel *et al.* are cleaved in the absence of the formation of a secondary structure;
- R. Youil *et al.*, "Screening for Mutations by Enzyme Mismatch Cleavage with T4 Endonuclease VII," *Proc. Natl. Acad. Sci. USA* (1995). This paper discloses a method for screening for mutations which utilizes a T4 resolvase enzyme to cleave mismatched heteroduplexes formed between wild-type and mutant DNA. This paper shows that cleavage of double-stranded DNA templates containing a single base mismatch by T4 endonuclease VII is influenced by DNA sequence (*i.e.*, the enzyme is not a structure-specific,

sequence- independent cleavage agent; in contrast, the enzymes used in the methods of the present invention are structure-specific, sequence-independent cleavage agents). The method requires the use of a double-stranded DNA template derived from two different sources of DNA (*e.g.*, a wild-type and mutant form of a gene). In contrast, the methods of the present invention do not require the use of a double-stranded DNA template derived from two different sources of DNA for the detection of mutations;

- European Patent Application No. 912202731.5 (Publication No. 0 482 714 A1) to Sullivan. Sullivan describes a *Thermus aquaticus* gene which encodes a thermostable DNA polymerase (*Taq*) with an altered N-terminus. This alteration is designed to provide improved expression of the *Taq* gene in *Escherichia coli*. In contrast to the present invention, Sullivan does not teach the cleavage of a nucleic acid substrate containing cleavage structures to generate a characteristic set of cleavage products;
- Murphy *et al.*, "Use of the 5' Noncoding Region for Genotyping Hepatitis C Virus," J. Infect. Diseases 169:473 (1994). Murphy *et al.* disclose identification of six hepatitis C virus (HCV) genotypes using restriction length fragment polymorphism (RFLP) of the 5' noncoding region of the HCV genome. The PCR-amplified 5' noncoding region sequences are digested with restriction endonucleases, and the restriction reaction products are separated by gel electrophoresis. Murphy *et al.* report that each of the six HCV genotypes exhibits a unique pattern of restriction cleavage products. The RFLP method of Murphy *et al.* is distinguished from the claimed method in that RFLP relies on the unique locations of restriction enzyme recognition sequences in the 5' noncoding region of the genome of different organisms. This is in contrast to the claimed method which relies on the formation of secondary structures and cleavage of those structures. The RFLP method of Murphy *et al.* does not

comprise either formation or cleavage of secondary structures in the nucleic acid substrate;

- Takada *et al.*, "HCV genotypes in different countries," Lancet 339:808 (1992). Takada *et al.* disclose the prevalence of different HCV genotypes in different countries. HCV-RNA encoding a portion of the NS5 domain of the HCV genome is amplified using reverse transcription polymerase chain reaction. The amplified cDNA fragments are genotyped by slot-blot hybridization with probes specific to each type of HCV or by restriction fragment length polymorphism analysis. The slot-blot method of Takada *et al.* is distinguished from the claimed method because the slot-blot method relies on binding of labelled oligonucleotide sequences to specific nucleotide sequences on the amplified cDNA fragments. In contrast, the claimed method relies on the formation of secondary nucleic acid structures followed by cleavage of those structures. The restriction fragment length polymorphism method of Takada *et al.* also differs from the claimed method. Restriction fragment length polymorphism relies on cleavage at sequence-specific restriction enzyme sites, rather than on cleavage of secondary structures, in the nucleic acid sequence;
- Belkum, "DNA Fingerprinting of Medically Important Microorganisms by Use of PCR," Clin. Microbiol. Rev. 7(2): 174-184 (1994). Belkum discloses the use of PCR fingerprinting for genetic typing of eukaryotic and prokaryotic microorganisms. Nucleic acid sequences are amplified using oligonucleotide primers which target repeat sequences, random sequences, or sequences occurring at multiple sites in the genome. The amplified nucleic acid products are separated by gel electrophoresis and banding pattern homologies are evaluated. The PCR fingerprinting method of Belkum is distinguished from the claimed method in that the PCR fingerprinting method relies on the location and distribution of repeat sequences, random sequences or sequences occurring at multiple sites in the nucleic acid substrate. In contrast, the claimed method

exploits the formation of secondary structures in the nucleic acid substrate. Additionally, whereas the claimed method comprises cleavage of the secondary nucleic acid structures, PCR fingerprinting does not comprise a cleavage step;

- Wilson *et al.*, "Amplification of Bacterial 16S Ribosomal DNA with Polymerase Chain Reaction," J. Clin. Microbiol. 28(9):1942-1946 (1990). Wilson *et al.* disclose a method for the detection of eubacteria using PCR amplification of the 16S rDNA. The 16S rDNA sequence is amplified using primers which are highly conserved among eubacteria and which are absent from eucaryotes, archaebacteria, or mitochondria. The PCR reaction products are separated by gel electrophoresis and the amplified sequences detected. Unlike the claimed method, the PCR method of Wilson *et al.* relies on the relative positions of certain conserved sequences with respect to each other, rather than on formation of secondary structures in the nucleic acid substrate. Moreover, in contrast to the claimed method in which the formed secondary structure is cleaved, the PCR method of Wilson *et al.* does not comprise such a cleavage step;
- Bingen *et al.*, "Use of Ribotyping in Epidemiological Surveillance of Nosocomial Outbreaks," Clin. Microbiol. Rev. 7(3):311-327 (1994). Bingen *et al.* disclose the use of ribotyping in combination with restriction fragment length polymorphism (RFLP) to differentiate microbial isolates. The RFLP method disclosed by Bingen *et al.* is essentially similar to the method used by Takada *et al.*, described *supra*. Both the Takada *et al.* method and the method used by Bingen *et al.* use restriction endonucleases to cleave the substrate DNA. However, instead of using DNA probes, Bingen *et al.* use an rRNA probe for the highly conserved bacterial rRNA genes (*rrn*) to detect the restriction fragments. Unlike the claimed method, the RFLP method of Bingen *et al.* relies on cleavage of the substrate nucleic acid sequence at restriction

enzyme sites, rather than on formation and cleavage of secondary structures in the nucleic acid substrate;

- U.S. Patent No. 5,455,170 to Abramson *et al.* provides DNA sequences encoding the wild-type and mutant forms of a nucleic acid polymerase from *Thermus species* Z05. This reference notes that the reverse transcriptase activity of the *Thermus species* Z05 polymerase has increased activity in the presence of Mn^{2+} as compared to Mg^{2+}. In contrast to the claimed invention, this reference does not teach a method for the cleavage of nucleic acids containing secondary structures. This reference is silent with regard to a teaching that the 5' nuclease activity of a DNA polymerase may function when Mn^{2+} is used in place of Mg^{2+}. Rather this reference speaks of the effect of Mn^{2+} upon the polymerization (*i.e.*, DNA synthetic) activity of a polymerase as measured by cDNA yields during reverse transcription; and
- Tabor *et al.*, "Effect Of Manganese Ions On The Incorporation Of Dideoxynucleotides By Bacteriophage T7 DNA Polymerase And *Escherichia coli* DNA Polymerase I," *Proc. Natl. Acad. Sci.* USA 86:4076-4080 (1989). This reference teaches that the efficiency of incorporation of dideoxynucleotides (*i.e.*, DNA synthetic activity) by T7 DNA polymerase and *E. coli* DNA polymerase I is more efficient when Mn^{2+} is used in place of Mg^{2+} in the reaction buffer. This reference is silent with regard to a teaching that the 5' nuclease activity of a DNA polymerase may function when Mn^{2+} is used in place of Mg^{2+}. In contrast to the claimed invention, this reference does not teach a method for the cleavage of nucleic acids containing secondary structures.

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

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Diane E. Ingolia

Diane E. Ingolia
Registration No. 40,027

MEDLEN & CARROLL, LLP
220 Montgomery Street, Suite 2200
San Francisco, California 94104
415/705-8410